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(57) Abstract

Methods of making and using D-antibodies and D-peptides are provided. The D-peptides or D-antibodies are analogs of ligands or receptors capable of specifically binding L-peptides, peptides containing both L-amino acids and D-amino acids, chiral and achiral non-naturally occurring peptides, and chiral and achiral non-peptide compounds. The D-peptides or D-antibodies are resistant to proteolysis in the gut and throughout the body and are less immunogenic than their L-polypeptide or L-antibody counterparts. Methods for producing such D-peptides or D-antibodies are provided.

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GENERATING D-PEPTIDES: METHODS AND COMPOSITIONS

INTRODUCTION

Background

There is a growing awareness of the need to produce and identify small molecules having pharmacological activity, including for example, agonists or antagonists of various cellular acceptor molecules, such as cell-surface receptors, enzymes or antibodies. Searching for small molecules that are useful as pharmaceuticals requires generating a collection of such molecules, screening the collection for molecules with physiological activity and identifying the structure of molecules providing a positive result in the screening step. The collection of small molecules can be generated using the combinatorial library approach. However, the prior art has not recognized the significance of using D-antibodies and D-peptides to screen such combinatorial libraries of small molecules for potential pharmacological activity and the ability to use mirror image transformations to create useful D-peptides and D-antibodies.

Nineteen of the essential twenty amino acids have the property of "chirality" or handedness. The only achiral essential amino acid is glycine. To describe a chiral compound, the prefixes D and L are used to refer to the configuration of the molecule around its chiral center. The chiral center of an amino acid is the alpha carbon, and whether an amino acid is of the D configuration or the L configuration depends upon the stereoisomeric conventions established by Emil Fisher. A chiral amino acid can exist as stereoisomers, which are identical chemical structures that are mirror images of each other. Both stereoisomers are often referred to as an enantiomeric pair, and a stereoisomer is often referred to as an enantiomer, which is a nonsuperimposable mirror image of the other stereoisomer/enantiomer.

All of the naturally occurring chiral amino acids exist in the L configuration, and are referred to generally as L-amino acids. The stereoisomer of each chiral amino acid in the L-configuration is referred to as a D-amino acid.

A D-amino acid is one which has a configuration corresponding to the D-stereoisomer of the two stereoisomers of glyceraldehyde, L-glyceraldehyde and D-glyceraldehyde. All D-amino acid, D-polypeptide or D-peptide stereoisomers that have the same stereo chemical configuration as D-glyceraldehyde are designated as D-, and those having the same configuration as L-glyceraldehyde are designated as L-.

Because the enzymatic reactions in ribosomal translation of polypeptides are stereospecific for L-amino acids, peptides consisting of all-D amino acids do not generally occur naturally. Accordingly, all naturally occurring proteins and polypeptides consist of L-amino acids, with the exception of certain antibiotics and bacterial cell wall proteins, which contain a limited number of D-amino acids introduced by specific enzymatic means or other post-translational modifications rather than by biosynthesis on the ribosome.

D-amino acids also occur naturally in proteins in man as the result of post-translational modification by racemases and as a result of spontaneous racemization of proteins with a long in vivo lifetime. See Helfman and Bada, PNAS, 72:2891-2894 (1975). Racemization is a naturally occurring process that over time, will convert naturally occurring L-amino acids into a racemic mixture of both L- and D- amino acids.

A limited portion of the structure of several approved peptide-like pharmaceuticals include a few D-amino acids. Such products include the widely known antibiotics Valinomycin, Gramicidin A, Gramicidin S, and also the vasopressin analog Desompressin (RPR), Lupron (Abbott), Synarel (Syntex), Sandostatin (Sandoz), SK&-110679 (Smithkline Beecham), and Decapeptyl (Ipsen-Beuafor/Akzo). Such structures are small and are not solely composed of D-amino acids.

Currently, D-peptides are made by chemical synthesis, using techniques that are well-known in the art. For example, D-peptides can be synthesized using stepwise addition of D-amino acids in a solid-phase synthesis method involving the use of appropriate protective groups. Solid phase peptide synthesis techniques commonly used for L-peptides are described by Meinhofer, Hormonal Proteins and Peptides, vol. 2, (New York 1983); Kent, et al., Ann. Rev. Biochem., 57:957

(1988); and Bodanszky et al., Peptide Synthesis, (2d ed. 1976), all of these references are incorporated by reference herein. D-amino acids for use in the solid-phase synthesis of D-peptides can be obtained from a number of commercial sources.

D-peptides and peptides that contain mixed L- and D-amino acids are known in the art. Also, peptides containing exclusively D-amino acids (D-peptides) have been synthesized. See Zawadzke et al., J. Am. Chem. Soc., 114:4002-4003 (1992); Milton et al., Science 256:1445-1448 (1992). Ligand analogs that are known in the art are small organic molecules, L-peptides, and modified L-peptides. However, D-antibodies that specifically bind receptors or ligands or substrates have not been described in the literature and there remains a need for such D-antibodies and D-peptides that are analogs of ligands and receptors as well as methods for their identification and production.

SUMMARY OF THE INVENTION

Accordingly, it is an object of the present invention to provide methods and compositions relating to analogs of biologically active peptide and protein, including but not limited to hormones and neuropeptides, wherein the analogs are comprised exclusively or essentially of D-amino acids and are biologically functional.

One aspect of the present invention concerns generating D-peptides and D-proteins composed entirely of D-amino acids which will interact with a natural or artificial L-peptide or L-protein target, such as a biological receptor in the body.

Novel compositions of matter comprising antibody-like entities comprised of D-amino acids (D-antibodies or D-peptides that are analogs of ligands or receptors) and engineered derivative forms, and a process for producing those novel antibody-like entities in such a way as to achieve required biological and pharmaceutical functions, are provided. The general method allows production of a molecular recognition surface (such as Van der Waal's and electrostatic surface) of a D-peptide (i.e. a polypeptide or protein composed entirely or largely of D-amino acids, including D-antibodies or fragments thereof) in such a way that it

will mimic the molecular recognition surface of a natural biological ligand composed of L-amino acids, while retaining the advantageous properties of D-peptides. Such D-peptides will be advantageously resistant to proteolysis in the gut, resistant to serum and tissue proteases, and are relatively immunologically inert. The D-antibodies of the invention or fragments thereof, including their antigen binding loops, or redesigned components thereof, will have increased resistance to proteolysis in the gut and throughout the body. Compositions comprising the D-antibodies and other D-polypeptides of the invention are contemplated. D-peptide analogs of L-antibodies which preserve the binding specificity of the antigen binding loops are possible, such that relatively small structures which are no-longer antibody-like in character essentially function as analogues of natural biological ligands. Alternative screening methods to refine the immunological approach are provided, including the phage-generation of FAb fragments that is known in the art and is an alternative to the standard monoclonal antibody method of generating an antibody to a specific antigen.

An additional advantage over the use of normal L-antibodies is that the antibody may be drastically modified including for example retaining only the FAb fragment in a D- analog or retaining the basic antigen recognition and binding loops placed on a scaffold (here, of D-amino acids) in the same orientation as in the original antibody. The design need not directly modify the antibody recognition region, but the resulting D-antibodies or D-polypeptides may differ considerably in form from antibodies. However, D-polypeptides are usually both resistant to proteolysis and much less immunogenic than the corresponding L-polypeptides.

In the preferred embodiment, monoclonal antibodies are raised against the D-amino acid sequences corresponding to the L-amino acid sequences of epitopes, domains or whole proteins where the L-amino acid sequences of such epitopes, domains or whole proteins are previously identified by experimental or computational means. A binding site on a protein ligand would be an example of typical interest as a structure comprising the aforesaid epitope, domain, or protein. The sequence of the monoclonal antibodies raised against the epitope domain or protein are then determined, and the whole antibody or any part thereof which

includes the antigen binding site or part thereof is synthesized as the D-amino acid sequence corresponding to the sequence or part of sequence in the monoclonal antibody. The resulting antibodies or subfragments thereof which are composed entirely of D-amino acids generally interact with the original natural, biological L-forms of the above mentioned epitopes, domains or whole proteins. Unlike humanized antibodies, heavy modification of the D-polypeptides of the invention, including reduced size of the peptide chain is possible without typically requiring design to maintain humanization, and the resulting D-polypeptide may be longer lasting *in vivo* than pharmacologically functional analogs of proteins, such as hormones and neuropeptides and natural antibodies.

In another aspect, the invention comprises the method described above, wherein the synthesis of a D-peptide corresponding to the monoclonal antibody comprises determination of the binding site of the monoclonal antibody and the L-amino acid sequence of said binding site and synthesis of a D-peptide that corresponds to the L-amino acid sequence or sequences of the binding sites of the monoclonal antibody, i.e. the D-peptide has the same amino acid sequence as the L-amino acid sequence of the binding site, except that the D-peptide has D-amino acids in place of the L-amino acids of the monoclonal antibody binding sites.

In yet another aspect, the invention provides synthesized D-antibodies comprising polypeptides or peptides comprised of a D-amino acid sequence that corresponds to an L-amino acid sequence of an L-antibody consisting of L-amino acids. The D-antibodies of the invention are comprised exclusively or essentially of D-amino acids or the corresponding enantiomers of amino acid analogs. Also, the D-antibodies of the invention may contain one or more of the achiral glycine amino acid residues.

The D-antibodies of the invention can include a receptor, a substrate binding site on an enzyme, an epitope of a receptor that interferes with ligand binding when an antibody is bound to the receptor, a ligand binding site of a receptor, a co-factor binding site on an enzyme and a sugar binding site on a protein. In another embodiment, the D-peptides can include a ligand for a receptor, a substrate for an enzyme binding site, a peptide hormone for a receptor, a non-peptide hormone for a receptor, a neurotransmitter for a receptor, a co-

factor for a co-factor binding site on an enzyme and a sugar for a sugar binding site on a protein.

As yet another aspect, methods of screening and molecular activity and processes which are the equivalent to producing a recognition surface in this manner whether involving biological manipulation or chemical synthetic methods, such as mass or combinatorial screening, are also within the scope of the invention. As described below, L-antibodies that are generated in response to D-peptide antigens can be produced using the known methods of monoclonal antibody production and phage-generation of FAb fragments.

DETAILED DESCRIPTION

Generally, the method of generating a D-peptide that binds to a ligand or receptor entails creating a D-version of the ligand or receptor, probing or screening a library made of L-peptides with the D-version of the ligand or receptor, detecting "hits" or L-peptides that bind to the D-version of the ligand or receptor and then synthesizing a D-version of the L-peptides. The D-version of the L-peptide is capable of binding to the L-version of the ligand or receptor. Such D-versions of the L-peptide are often referred to herein as D-peptides or D-antibodies. These steps can be conducted sequentially or repeated (such as the screening step) before proceeding to the next step. Further it will be apparent that completion of only some of the steps will facilitate the design of active molecules, such as therapeutics.

Usually, the first step is to choose a protein target (or non-peptide ligand), such as a receptor, or enzyme to which one wishes to design a novel D-peptide ligand. Typically, such a ligand D-peptide will provide either inhibition of the normal function as an antagonist, or in some cases of the normal function activation as an agonist. The target proteins or protein components (such as a ligand binding site) are synthesized in their mirror image form, by making them of D-amino acids (D-polypeptides). The resulting molecules may be referred to D-polypeptides, such as D-receptors, D-enzymes, or D-hormones, parts of which correspond in sequence to normal L-receptors, L-enzymes and L-hormones, respectively. Usually the amino acid sequences of the D-version and the L-version

of such molecules are identical except for the fact that they are mirror images of each other. The term D-receptor will be used to refer to all protein targets whether D-receptors, D-enzymes, D-hormone or any other D-protein, or to parts of such proteins, for which an agonist, antagonist, or any other novel ligand is desired. As one typical embodiment, a ligand binding domain of a D-receptor would be made by first looking at the amino acid sequence of the ligand binding domain of the natural receptor, and then one may re-synthesize the same sequence using D-amino acids instead of L-amino acids. Ligand binding sites with molecular recognition surfaces contacting at least two surfaces of the ligand are preferred, especially when the recognition surfaces are less than 100 amino acids apart in the amino acid sequence of the protein.

Usually, the second step is to perform combinatorial screening of an L-peptide library by probing with the D-receptor or D-polypeptide. Sometimes it will be desirable not to use the first step if the target is already known. Combinatorial screening permits the creation of a diverse set of peptides that can be then converted into D-peptides. Alternatively, immunological production of antibodies or recombinant production of antibodies can be used to create a diverse set of L-peptides that can be converted into the corresponding D-peptides. Synthetic peptide libraries can be prepared combinatorially in advance, and then screened against the D-receptor. One advantage of synthetic peptide libraries is that they provide greater chemical diversity when non-naturally occurring amino acids are used, especially amino acids with the same charge as naturally occurring amino acids but differing in the distance that the charge is located from the peptide backbone. Alterations in distance can be accomplished with 1, 2, or 3 atom extenders of the negative or positive charge, preferably carbon atoms are used. Recombinant peptide libraries can be used as well, as known in the art.

One type of preferred combinatorial library is the phage display D-peptide approach. Typically, one would first prepare a bacteriophage display with normal L-peptides using methods known in the art. Alternatively, other recombinant libraries could be used that rely on plasmids to produce L-peptides. Here the combinatorial presentation of many different peptides or proteins is implemented indirectly, by random mutation or partly random mutation or combinatorial

selection of nucleotide segments of the DNA expressing the proteins. Preferably in recombinant libraries, such as phage display libraries, a short section of DNA encodes a mutated region that will become part or all of the L-peptide. Preferably, the mutated region is not randomly mutated and does not contain all possible amino acid sequences for a 3 to 10 or a 5 to 8 amino acid mutated region. Instead mutations are selective and usually introduce at predetermined amino acid positions, conservative mutations, such as swapping polar amino acids for different polar amino acids, negatively charged amino acids for different negatively charged amino acids and hydrophobic amino acids for different hydrophobic amino acids. Such mutated regions be used with synthetic peptide libraries and can are preferably used with D-antibody phage display.

Bacteriophage libraries are inexpensive and easy to manipulate, Bacteriophage display or "phage display" is widely used. It was originally developed as "fusion phage" technology (S.F. Parmley and G.P. Smith, Gene, 73:305-318, 1988, herein incorporated by reference) to describe the chimeric nature of coat proteins displaying random amino acids at their N-termini. The earliest phage display libraries, developed as a technology for more general screening purposes, appeared in a number of laboratories in 1990 and were typically implemented by cloning a synthetic piece of DNA into gene III of an fd or M13 filamentous bacteriophage (J.K. Scott and G.P. Smith, Science **249**:386-390, 1990; J.L. Devlin, L.C. Panganiban, P.E. Devlin, Science **249**:404-406, 1990; S.E. Cwirla, E.A. Peters, R.W. Barrett, W.J. Dower, Proc. Nat. Acad. Sci. USA **87**:6378-6382, 1990). In these cases one may also display, not just combinatorially generated peptide segments but regions on protein domains from other sources to stabilize the protein or aid in its display. The gene or gene segment for such regions is inserted into the bacteriophage DNA and expressed as a domain with variable sequence content at the surface of the bacteriophage protein. Polymerase Chain Reaction amplification of the bacteriophage plaque followed by DNA sequencing identifies the genes which have generated peptide sequence binding to a target receptor.

A further advantage of this combinatorial screening is to increasingly refine the screening with further cycles of screening. Preferably, additional cycles of

screening offer higher stringency conditions to select for tighter binding, i.e. a lower apparent Kd. Higher stringency conditions can be accomplished by increasing the temperature, lowering or increasing the ionic strength, increasing the concentration of chaotropic agents and the like, or a combination thereof. For example, one can infect cells with an affinity purified population of phage so that the next selection by affinity uses up to a million copies of the original selection allowing a bacteriophage to be retrieved and amplified. In such a typical case, the method allows access to at least 10^8 different peptides in a tube of approximately 1 ml volume.

Usually, there is a third step where the L-peptides are detected and then sequenced or identified as to which sequence they represent using a detection or identification means, many of which are known in the art for combinatorial libraries. In using combinatorial peptide libraries, the binding or enriched peptides respectively may be subjected to amino acid sequencing e.g. by Edman degradation, or identified by other means. For example, "hits" may identified by position on a grid on which the combinations were generated in a controlled manner, or by chemically labeled tags including radio-labeled tags, by unique linked nucleic acid labels, by their final mass as determined by mass spectrometry (see PCT/US95/03355, which is herein incorporated by reference), by iterative resynthesis and screening of smaller subpools or submixtures (see Zuckermann et al., J. of Medicinal Chem., 37(17): 2678-2685 (1994)(incorporated by reference herein), or by any combination of these or other known techniques. In using bacteriophage display, the sequences are usually deduced by amplification and inspection of the DNA sequence coding for the peptide of interest.

Usually, there is a fourth step where the L-peptide or L-protein sequences are noted and the D-peptides or D-proteins are then made with the same amino acid sequence, using D-amino acids rather than L-amino acids. These D-peptides or D-proteins correspond to the mirror images of the selected combinatorial peptides. Such molecules can now interact with the original L-target receptor having the amino acid sequence corresponding to the D-receptor that was made for the screen. Such receptors again include for present purposes receptors proper,

enzymes, hormones and other proteins against which one may wish to make a D-peptide ligand.

An important aspect of the invention is the display of L-antibodies or parts thereof such as Fab fragments by cloning into the phage, not only to refine the binding site (recognition loops) to the target, but to refine the frame, particularly the Fab fragment bearing the antigen binding site. Particularly but not exclusively, all the peptides and protein displayed on the phage will contain at least the antigenic binding loops of the monoclonal antibodies raised against the original D-antigen, and which will carry the recognition in D-form to interact with the original L-form of the antigen, say a natural receptor in the body. As stated above the invention includes that these loops may be modified by phage display for refinement purposes, and this also includes that a degree of design may be carried out so that refinements of the antigenic binding site sequences are raised in the phage. The modification envisaged by phage display include extensive modification of the frame, replacement by non-antibody frames (folds of other proteins) to carry the recognition loops, and a high degree of reduction in size including removal of the frame possibly including insertion of Cystine cross links. Modification of antibody components and selection by phage display is known in the state of the art but without regard to D-antibodies, and is known as use of "semisynthetic antibody libraries".(C. Barbas et al., Proc. Nat. Acad. Sci. USA, 89,4457,1992). It is important to note that these modifications and the ability to perform them with beneficial effect on the final product have a special and unprecedented significance in regard to this present invention described herein which results in assigning a required recognition surface to a D-peptide or D-protein. As described above the D-Antibodies or fragments thereof will be resistant to proteolysis and less immunogenic, an effective form of humanization. Thus, the antibodies will retain humanization in D-form even when drastically modified. This includes any forms generated by phage display and made of D-amino acids. This natural humanization is not the case for drastic modification in the usual application of phage display of semisynthetic antibody libraries. Hence this present invention allows that the Fab frame can be progressively reduced in size with mutations included and screened so that the activity is selected to be retained. Reduction of size in a single jump

might well lose important interactions with the frame, causing the binding loops to become distorted. It was noted in a computer simulation study (V.P.Collura, P.J.Greany and B.Robson, Protein Engineering, 7, 2211-223, 1992), of the antigen recognition loop H2 from Fab fragment McPC603, that there are important interactions between the antibody recognition loops and the rest of the Fab fragment. Alternatively, the antigen recognition loops themselves, being three on the heavy chain and three on the light chain, may be presented and refined for binding, to be synthesized as D-peptides without a supporting molecular scaffold, individually or joined, for example with oligoglycine spacers. Alternatively they may be presented on a scaffold other than an Fab fragment or other antibody component, in which the active loops are replaced by three selected antibody recognition loops. Typically these will be the loops identified in binding studies as binding most strongly to the target or its analog, but are expected to be the three loops of the heavy chain as in most antibodies where studies have been carried out, these have more extensive interaction with the antigen. Similarly, the two larger loops of the heavy chain may be selected on occasion without the third smaller loop. In seeking a protein scaffold, engineering may be required including using one or more loops in the retro (backwards) sequence direction. In all the above examples, the initial proposed structures are typically to be refined by phage display.

Optionally, these reagents may need refinement as a drug, by rational design by exploring a structure-activity relationship of analogues, or by modeling studies at the receptor where this is of known structure, and may optionally include for design and refinement purposes comparison with retroinverso forms of known natural or discovered or designed L-peptide ligands and also optionally by comparison with the receptor sequence. Further refinement may be required for enhanced oral delivery and availability and improved pharmacokinetic properties. Further chemical modification including added groups including for example extension at either terminus with additional amino acid residues may be required. One may also require a presentation medium such as a liposome system to be used. Peptide extensions for targeting and cell entry can include for example L-amino acid based antibodies and L-hormones preferably of long half life.

Peptide and protein extensions and additions which may be made of D-amino acids and still be active in D-forms include magainins, cecropins and other lytic peptides, viral cell entry peptides, endosome escape peptides of viral origin, peptides of for example 1gG3 or milk protein origin which may cross the blood brain barrier.

In one embodiment antibodies composed exclusively or essentially of D-amino acids interact with peptides and proteins made of L-amino acids in effectively the same way as the corresponding L-antibodies made of L-amino acids will interact with peptides or proteins made of D-amino acids. By preparing monoclonal antibodies against the D-peptide or D-protein sequences otherwise identical to L-peptide or L-protein sequences of the required pharmacological target, and then making the D-antibody corresponding to the monoclonal antibody, matter is effectively reflected twice through the plane of a hypothetical mirror, leaving the binding site of the D-antibody as a molecular recognition analog of the endogenous or natural pharmacological target.

The pharmacological target can be any natural endogenous or other biological or non-natural ligand or receptor, including a hormone, neuropeptide, virus particle, other biologically active peptide, or enzyme. L-polypeptide or L-protein sequences of the target may represent an epitope or set of epitopes in or near the binding site of a receptor or other protein target, or the subdomain or domain of the receptor or other target, or the whole or part of the receptor or other target. The L-polypeptide or L-protein amino acid sequences are used to generate the corresponding D-amino acid sequence, which is the same sequence as that of the L-polypeptide or L-protein sequence of the target amino acids differing only in enantiomeric form and comprising D-amino acids. Not all amino acids need to be converted from L to D form so long as they do not alter the antigenicity and immunogenicity of the synthesized D-peptide antigen and do not alter the specificities of the resulting D-antibody.

METHOD OF MAKING D-ANTIBODIES AND D-PEPTIDES THAT ARE ANALOGS OF LIGANDS OR RECEPTORS

The present invention provides a method for identifying a D-antibody or a D-peptide that is an analog of a ligand or a receptor comprising: selecting the ligand or a receptor; determining the L-amino acid sequence comprising either the ligand or the ligand binding site of the receptor; synthesizing a D-peptide corresponding to the L-peptide or L-polypeptide comprising the ligand or the ligand binding site of the receptor; preparing a monoclonal antibody to said D-peptide; synthesizing a D-antibody comprising a D-amino acid sequence corresponding to an L-amino acid sequence of the monoclonal antibody; and assaying said D-antibody for specific binding to the ligand or the ligand binding site of the receptor. The D-peptide or D-polypeptide corresponding to the L-peptide or L-polypeptide has the same amino acid sequence from the carboxy terminus to the amino terminus as the L-peptide or L-polypeptide, except that the L-amino acids are replaced with their corresponding D-stereoisomers.

This method can be used for any ligand or receptor, so long as the molecule has a stereochemical configuration and the enantiomer of the molecule can be prepared. Thus, the ligands can be non-peptides, non-naturally occurring ligands, and peptides comprising a mixture of D- and L-amino acids. In general, any epitope of a ligand or receptor might be synthesized using D-amino acids and attached to a carrier molecule to render it immunogenic. In such a case, the D-epitope may be regarded as a hapten. A D-protein in excess of approximately 35 residues may not always be immunogenic without such a carrier molecule, since it is unlikely to be cleaved an presented on major histompatibility antigen to the T-cell receptor. The D-peptides or proteins made from antibodies raised against the D-epitopes may for example include activation of a receptor by inducing dimerization of receptor proteins or oligomerization generally.

In the case of non-peptide ligands or receptors for which mirror-image analogs are desired, the method is modified such that the enantiomer of the non-peptide ligand or receptor is synthesized and used to generate an antibody that specifically binds to it. Then, the L-amino acid sequence of the antibody or a

portion thereof, such as its Fab fragment, is determined, and from the L-amino acid sequence the corresponding D-antibody is synthesized.

The method can also be applied to achiral ligands or receptors or molecules. For achiral ligands or receptors or molecules, the method comprises using the achiral ligand, receptor or other molecule to generate an L-antibody; determining the amino acid sequence of the L-antibody or a portion thereof, such as the Fab fragment; synthesizing the corresponding D-antibody; and assaying the D-antibody for specific binding to the achiral ligand, receptor or other molecule.

The selection of the chiral or achiral, natural or non-naturally occurring, ligand or receptor or other molecule to which a D-antibody is desired for binding, is accomplished using information about such molecules in the literature as well as any information regarding the amino acid sequence, if any, of such molecules. The selection of a ligand binding site of an L-polypeptide or naturally occurring protein is accomplished by reference to the literature regarding amino acid sequences of known ligand binding sites, receptors or ligands. If any such molecules are peptides that have not yet been sequenced, one of ordinary skill can sequence the peptides using well-known peptide sequencing methods.

The synthesis of a D-polypeptide antigen that corresponds to an L-amino acid sequence of a peptide ligand, receptor or other molecule, is accomplished using chemical synthesis. The D-polypeptide antigen is synthesized, preferably using known solid phase stepwise Merrifield-type peptide synthesis techniques developed for L-peptide synthesis, but in this case using D-amino acids or protected D-amino acids in the stepwise synthesis. Other methods of synthesizing D-polypeptide antigens are contemplated, including covalent bonding of monomers that were produced via stepwise synthesis. The means of synthesizing the D-polypeptide antigens and other D-polypeptides or D-antibodies of the invention are known in the art, and the invention can be practiced using methods of D-polypeptide synthesis that are yet to be developed.

Generally, the solid-phase synthesis of D-peptides comprises: attaching a protected D-amino acid to an inert solid support through the unprotected carboxyl or amino group of the D-amino acid; selectively removing the protecting group on the amino or carboxyl group of the first D-amino acid; introducing the next D-

amino acid having the appropriate amino or carboxyl group protected and reacting it under conditions that permit formation of an amid linkage between the second D-amino acid and the first D-amino acid already attached to the solid support. The protecting group on the amino or carboxyl group of the second D-amino acid is then selectively removed. This procedure is repeated for each successive addition of a D-amino acid to the synthesized D-peptide. After the D-peptide has been completely synthesized, protective groups if any remaining on the D-peptide are removed, and the chemically synthesized D-peptide is cleaved from the solid support. In order to make a large, D-peptide, the chemically synthesized D-peptides can be chemically ligated using methods known in the art.

Once the D-polypeptide antigen is synthesized, it is used to produce antibodies. The antibodies to be produced can be either monoclonal antibodies or phage-generated FAb fragments, both methods are known in the art. As discussed above, a carrier molecule may be required to render the D-peptide or protein immunogenic, particularly since it may not be able to cleave the D-molecule for presentation as major histocompatibility complex antigens to the T-cell. Such molecules are available commercially as kits already primed with chemical groups to join to the peptide epitope. Proteins typically available include Keyhole Limpet Hemocyanin and Bovine Serum Albumin. The preparation of monoclonal antibodies to the D-polypeptide or D-peptide is accomplished using standard methods for production of monoclonal antibodies specific for a desired antigen. Phage-generated peptides are described in PCT/US91/04384 (Dower et al., filed June 19, 1991); PCT/US91/02989 (Dower et al., filed May 1, 1991); PCT/US92/08879 (Schatz et al., filed Oct. 15, 1992); PCT/US94/05796 (Aldwin et al., filed May 23, 1994); U.S. Patent No. 5,491,074 (Aldwin et al., filed May 24, 1994); and PCT/FR95/00127 (Sodoyer et al., filed Feb. 2, 1995), all of which are incorporated herein by reference. The monoclonal antibodies or phage-generated FAb fragments are screened using methods known in the art for specific binding to the D-polypeptide antigen. The selected antibody or a portion of the antibody, such as the modified Fab fragment or a single-chain Fv or disulfide-bonded Fv fragment is then isolated and sequenced. Fv fragments are the smallest functional moieties of antibodies required for binding of an antigen. Reiter et al.,

Protein Engineering, 7(5):697-704 (199). The L-amino acid sequence of the antibody or portion representing a binding site, Fab fragment, single-chain Fv fragment or disulfide-bonded Fv fragment is then used to determine the corresponding D-amino acid sequence of a D-antibody of the invention. The D-antibody of the invention, which includes D-amino acid sequences corresponding to an entire L-antibody or portions thereof, including the Fab fragment, the single-chain Fv fragment and the disulfide-bonded Fv fragments, is synthesized as described above.

In the case of the use of phage display of antibody, it is known that antibodies are or parts of antibodies displayed can be refined by phage display in their binding recognition loops and in part of the supporting antibody fold; such antibodies are termed "semisynthetic" (C.F.Barbas et al., Proc. Nat. Acad. Sci. USA 89-4457, 1992). Normally modification to the frame of the antibody or Fab fragment confers no advantage in clinical use since humanization will most typically be lost. However, as described above the principle significance of the invention is that the protease-resistant properties and reduced immunogenicity of D-antibodies implies a degree of built-in humanization, such that modification to the frame is not in general a restriction. Consequently, the invention allows for all such modifications to the frame such as would normally risk loss of humanization, including reduction of size of the frame as discussed above, in the practice of phage display, and including progressive reduction of the size of the antibody frame in phage display. This includes reduction in steps with enrichment by passing the cloned Fab head with randomized amino acids by phage display on progressively smaller frames, such that screening is performed at each size reduction step to retain activity. In contrast, too large a size reduction in a single step would risk total loss of activity due to too large a change of interactions between loop and frame.

This invention greatly broadens the scope of application of D-proteins by readily allowing production of biologically functional forms. By taking the sequence, or portions thereof of genes which express proteins as the starting point, the invention also provides a means for more direct conversion of information in the human genome into pharmaceutical products.

The D-antibodies of the invention, which include both D-analogs of complete antibodies as well as D-analogs of portions of antibodies, can be used in therapeutic compositions, as agonists or antagonists or catalysts. Also, the D-antibodies can be used to specifically bind target cells that express specific antigens, such as viral or bacterial antigens. The D-antibodies, which are not only D-antibodies but also D-peptide analogs of Fab fragments or other portions of L-antibodies, have several advantages over the corresponding L-antibodies. First, because biological systems and proteins and enzymes are generally stereospecific for L-polypeptides or L-amino acids, the D-antibodies of the invention are resistant to proteolysis in the gut and throughout the body. Second, since D-peptides are generally less immunogenic than their corresponding L-peptides, the D-antibodies of the invention are less likely to cause an immune response in a human host, which is an important characteristic for therapeutic compositions (U.S. Provisional Application 60/005,508 filed October 10, 1995 and U.S. Provisional Application 60/014,433, are herein incorporated by reference).

D-Peptides That Are Analogs of Ligands or Receptors

The D-antibodies of the invention are essentially D-peptides that are analogs of ligands or receptors that are capable of binding to peptide or non-peptide, natural or non-naturally occurring, chiral or achiral molecules.

The D-antibodies can be designed to bind to either receptors or ligands or any chiral or achiral, natural or non-naturally occurring molecules. When a D-antibody capable of specifically binding to a ligand is desired, then it is produced according to the methods described herein, using the ligand as either the template from which an amino acid sequence is obtained (if the ligand is a polypeptide) or as the antigen against which L-antibodies, whether monoclonal or phage-generated, are raised. Ligands include naturally occurring polypeptides or peptides, non-peptides such as hormones, non-naturally occurring peptides or non-peptides, and chiral or achiral compounds. When a D-antibody that specifically binds a receptor is desired, it is produced according to methods described herein. In one embodiment, the receptor or a portion thereof is used as the original polypeptide from which an L-amino acid sequence can be determined and the corresponding

D-polypeptide antigen synthesized. Use of combinatorial libraries are well known in the art. See PCT/IB95/00560 (Hodges et al., filed June 13, 1995) and PCT/US95/03355 (Benkovic et al., filed March 23, 1995) both of which are incorporated by reference herein.

Ligand generally refers to a member of a ligand binding pair, i.e. a ligand and a receptor. A portion of the ligand or surface of the ligand specifically binds a portion of a receptor or a surface of a receptor. Most often a ligand can exert a biological effect, i.e. a biological ligand. Generally, the ligand's overall structure or molecular weight is smaller than the receptor, but this is not a necessary condition. A ligand is often a L-peptide or L-polypeptide, but other non-peptide molecules, including steroids, co-factors, neurotransmitters, neurotransmitter analogs, non-peptide hormones, non-peptide hormone analogs, and nucleotides, nucleosides and sugars and modified forms of non-peptide molecules are contemplated as ligands. Non-naturally occurring and achiral ligands are also contemplated. Usually, ligands will not include D-polypeptides or D-amino acids. Usually, the affinity of the ligand for the receptor (apparent Kd at a relevant temperature, ionic strength, and pH, such as at a human physiological condition) is less than 1mM, preferably 1 pM to 100 μM or less than 100 μM, more preferably 10 pM to 1 μM or less than 1 μM, and most preferably 100 pM to 100 nM or less than 100 nM. A D-ligand refers to a ligand with a D- configuration, usually a non-peptide.

Receptor generally refers to a member of a ligand binding pair. A receptor need not be considered a biological receptor with a specific biological function. Instead, receptor refers to a member of the ligand binding pair with a molecular recognition surface that binds, usually non-covalently, to the ligand. The receptor will have at least one ligand binding site with such a surface. Generally, the ligand binding site will be solvent accessible, preferably water accessible. The ligand binding site will often be composed of L-amino acids linked together by peptide bonds, such as a biological receptor for a peptide hormone. In other instances, the receptors might be an organic molecule, such as crown ether, or a nucleic acid, such as DNA.

D-antibody refers to an antibody or fragment thereof that includes a D-amino acid sequence, including modifications to maintain the fragment or separate fragments in the conformation that they would have in the entire D-antibody (and hence also the mirror image to the conformation that the corresponding L-peptide segments would have in the monoclonal L-Antibody). The fragments will typically be the binding recognition loops of the antibodies that are involved in direct interaction with antigen, there being three such loops on the heavy chain and three such loops on the light chain of the antibody. For example, it has been noted that the conformation of the antigen-combining loop H2 from Fab McPC603 V.P. Colura, P. G. Greany, and B.Robson, Protein Engineering 7:221-233, 1994) depends critically on interaction with the rest of the Fab antibody fragment. Modifications can be of various types. Generally, a D-antibody amino acid sequence will be entirely made of D-amino acids. In some instances it will be preferable to include L-amino acids in the amino acid sequence of a D-antibody. In particular, after reading the methods of the invention herein, it will be apparent that the molecular recognition surface of a D-antibody will be primarily, if not entirely, composed of D-amino acids, while the other regions of a D-antibody, if present can be primarily, if not entirely, composed of L-amino acids, such as the non-variable regions of a monoclonal antibody. Additionally, D-antibodies can include single chain FAbs, or any subfragments or analogues thereof, where the heavy and light chains are joined end-to-end via a flexible linker, and mini-bodies, where the heavy and light chains are joined, preferably by a disulfide or similar bond by the introduction of two residues like cysteines, one in each chain. See Reiter et al., supra. Preferably the ligand binding site will be made of at least 90% D-amino acids, more preferable at least 95%, and most preferably 96% to 100% or 100%; wherein the percentage is calculated as the number of D-amino acids divided by the total number of amino acids in a sequence, multiplied by 100.

A D-amino acid sequence is normally used for making the protein (or peptide) ligand or receptor in the process of making a D-antibody that resembles the receptor or the ligand. When an analog to a protein ligand is desired, the D-antibody is made using the D-amino acid sequence of the protein ligand. When an analog to a protein receptor is desired, the D-antibody is made using the D-amino

acid sequence of the protein receptor. Such D-amino acid ligands and receptors can be considered a D-polypeptide antigen. Usually the D-amino acid sequence of either the protein receptor or ligand is composed entirely of D-amino acids, but substitution of L-amino acids is tolerated in regions of the protein that do not adversely alter the Kd by more than two orders of magnitude and more preferably one order of magnitude compared to an all D-amino acid protein ligand or receptor.

D-antigen refers to antigen with a D- configuration.

L-antibody refers to antibody made from L-amino acids, including those found in nature, made by immunizing mammals, made by phage and other methods known in the art for making truncated or modified antibodies, minibodies, including, but not limited to single chain antibodies or immunoreactive fragments thereof.

Detection Methods

The invention also provides for detection methods. As the antibodies and peptides of the invention provide for peptidase resistant molecules, such compounds of the invention are particularly suited for detection of analytes. Generally, the method of detecting an analyte comprises contacting the analyte with a D-antibody, and detecting a complex of the analyte and D-antibody. Many variations in the method of detection can be accomplished with the antibodies of the invention. The antibodies of the invention can be used for instance in ELISA assays and separation methods can be applied comprising an additional step of separating the complex from unbound D-antibody. Washing steps are not required if complementation assays, as known in the art, are used that change the rate of production of a detectable signal. Such assays can be conducted using a kit containing the necessary assay components.

A myriad of analytes can be detected using the antibodies of the invention and affinity selection for purification of the desired analyte(s). Primarily analytes will be those for which an antibody can be generated by methods known in the art at the time of the filing of this application and those methods later discovered. Generally, the analyte is a ligand for a receptor, a substrate for a binding site on

an enzyme, a peptide hormone, a non-peptide hormone, a neurotransmitter, a co-factor, or a sugar.

The antibodies of the invention can be labelled if so desired by covalent or non-covalent means to facilitate detection. Such labels include enzymes capable of generating a detectable signal, fluorescent compounds (including FITC), radioactive atoms (including C14 and I125), biotin, and avidin. Preferably, labels are attached at a region of the antibody that does not include the ligand binding site or at the C- or N- terminus. Labels can also include toxins to kill cells or inhibit cell proliferation.

The antibodies of the invention can also be used as biosensors in either using enzymatic or electrical detection methods or a combination of the two. Ligand specific electrodes can be generated using the antibodies of the invention as taught in the art for other biological molecules that bind ligands, particularly antibodies.

Compositions

The invention also includes pharmaceutical compositions and methods of modulating biological conditions. For instance antibodies of the invention can be used to inhibit ligand binding to a receptor by contacting an effective amount of D-antibody to a receptor, thus blocking the ligand from binding to the receptor. Usually, the amount will vary from 1 μg to 100mg, more preferably from 10 μg to 10mg or more than .01 mg, and most preferably from .1 mg to 10 mg.

Pharmaceutical compositions comprise a physiologically suitable carrier and the compounds of the invention. Such carriers include buffers and coatings as known in the art.

All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

The invention now being fully described, it will be apparent to one of ordinary skill in the art that many changes and modifications can be made thereto without departing from the spirit or scope of the appended claims.

WHAT IS CLAIMED IS:

1. A D-antibody comprising a polypeptide comprised of a D-amino acid sequence that corresponds to an L-amino acid sequence of an L-antibody consisting of L-amino acids.

2. The D-antibody of claim 1 wherein said L-antibody is a monoclonal antibody or a phage generated antibody fragment that specifically binds a D-antigen made from D-amino acids or D-ligand.

3. The D-antibody of claim 2 wherein said D-antigen is a D-polypeptide selected from the group consisting of a receptor, a substrate binding site on an enzyme, an epitope of a receptor that interferes with ligand binding when a receptor antibody is bound, ligand binding site of a receptor, a co-factor binding site on an enzyme and a sugar binding site on a protein.

4. The D-antibody of claim 2 wherein said D-antigen is selected from the group consisting of a ligand for a receptor, a substrate for a binding site on an enzyme, a peptide hormone, a non-peptide hormone, a neurotransmitter, a neurotransmitter analog, a steroid, a steroid analog, a co-factor, and a sugar.

5. The D-antibody of claim 2 wherein said monoclonal antibody is a modified FAb fragment.

6. The D-antibody of claim 5 wherein said monoclonal antibody is comprised entirely of D-amino acids.

7. The D-antibody of claim 3 wherein said monoclonal antibody preferentially recognizes an epitope on a cancer cell compared to a non-cancer cell.

8. The D-antibody of claim 3 wherein said monoclonal antibody recognizes an epitope on a virus.

9. The D-antibody of claim 6 wherein said monoclonal antibody has a label attached.

10. The D-antibody of claim 9 wherein said label kills cancer cells.

11. A method of making a D-peptide that is an analog of a ligand comprising:

1) selecting a L-amino acid ligand binding site or a L-ligand,

2) synthesizing a D-amino acid polypeptide that corresponds to said ligand binding site or a D-ligand,

3) preparing monoclonal antibodies to said D-amino acid polypeptide or said D-ligand, and

4) synthesizing a D-antibody comprising a D-amino acid sequence corresponding to at least a portion of said monoclonal antibody.

12. The method of claim 11 wherein said preparing comprises determining an amino acid sequence of said monoclonal's antigen binding site known to bind an antigen.

13. The method of claim 12 wherein said preparing further comprises injecting subcutaneously or administering orally said D-amino acid polypeptide into an appropriate mammal.

14. A method for identifying a D-peptide that is an analog of a peptide or polypeptide comprising:

1) selecting a L-amino acid ligand binding site,

2) synthesizing a D-amino acid polypeptide that corresponds to said ligand binding site,

3) preparing monoclonal antibodies to said D-amino acid polypeptide,

4) synthesizing a D-antibody comprising a D-amino acid sequence corresponding to at least a portion of said monoclonal antibody, and

5) testing said D-antibody for specific binding activity of a receptor of said peptide or polypeptide.

15. The method of claim 13 wherein said testing is selected from the group consisting of competitive binding assays, cell culture competitive binding assays, steroid receptor binding assays using gene activation, and displacement assays.

16. A method of detecting an analyte comprising:

1) contacting said analyte with a D-antibody, and

2) detecting a complex of said analyte and said D-antibody.

17. The method of claim 15 further comprising an additional step of separating said complex from unbound D-antibody.

18. The method of claim 15 wherein said analyte is selected from the group consisting of a ligand for a receptor, a substrate for binding site on an enzyme, a peptide hormone, a non-peptide hormone, a neurotransmitter, a co-factor, and a sugar.

19. A complex comprising a D-antibody and an analyte.

20. The complex of claim 19 wherein said analyte is a L-polypeptide antigen.

21. A method of inhibiting ligand binding to a receptor comprising contacting an effective amount of D-antibody to a receptor when said ligand is present.

22. A method of identifying an L-peptide corresponding to a D-peptide that is an analog of a ligand to an L-amino acid ligand binding site comprising:

1) screening at least one L-peptide with a D-polypeptide that corresponds to said L-amino acid ligand binding site, and

2) determining the binding of said at least one L-peptide to said D-polypeptide.

23. The method of claim 22, further comprising the step of:

3) synthesizing a D-peptide comprising a D-amino acid sequence that corresponds to at least a portion of an L-amino acid sequence of said at least one L-peptide,

wherein said at least one L-peptide binds to said D-polypeptide.

24. The method of claim 22, wherein said L-amino acid ligand binding site is a biological receptor.

25. The method of claim 22, wherein the amino acid sequence of said L-amino acid ligand binding site is identical to the amino acid sequence of said D-polypeptide, except that said D-polypeptide is made entirely of D-amino acids.

26. The method of claim 23, wherein said D-peptide is a D-antibody.

27. The method of claim 22, wherein said at least one L-peptide is at least one L-FAb fragment.

28. The method of claim 27, wherein said at least one L-FAb fragment is phage display library of L-FAb fragments.

29. The method of claim 28, wherein said phage library of L-FAb fragments contains fragments of progressively smaller L-FAb fragments, wherein no F-Ab fragment is less than 4 amino acids and each fragment is identical in amino acid sequence to at least one portion of at least one L-Ab fragment.

30. The method of claim 29, wherein said L-amino acid ligand binding site is a biological receptor.

31. The method of claim 30, wherein L-FAb fragments have a mutated region 3 to 10 amino acids in length containing selective amino acid replacements.

32. The method of claim 22, wherein said at least one L-peptide is at least ten L-peptides.

33. The method of claim 32, wherein said at least one L-peptide is a combinatorial library.

34. The method of claim 32, wherein said combinatorial library is a phage display library.

35. The method of claim 32, wherein said phage display library encodes peptides 4 to 20 amino acids in length and each peptide has a mutated region.

36. The method of claim 35, wherein said D-polypeptide is selected from the group consisting of a receptor, a substrate binding site on an enzyme, an epitope of a receptor that interferes with ligand binding when a receptor antibody is bound, ligand binding site of a receptor, a co-factor binding site on an enzyme and a sugar binding site on a protein.

37. The method of claim 33, wherein said combinatorial library comprises L-peptides ranging from 20 to 120 amino acids in length and no more than 50,000 peptides in complexity.

38. The method of claim 37, wherein said screening step comprises repeating said screening step at a higher stringency conditions to select for higher affinity binding of said L-peptides to said D-polypeptide.

39. The method of claim 38, wherein said L-peptides have an apparent Kd of 10 μM or less.

40. The method of claim 39, wherein said L-peptides have an apparent Kd of .01 μM or less.

41. The method of claim 40, further comprising the step of:

3) synthesizing a D-peptide comprising a D-amino acid sequence that corresponds to at least a portion of an L-amino acid sequence of said at least one L-peptide,

wherein said at least one L-peptide binds to said D-polypeptide

42. A method of generating a L-peptide corresponding to a D-peptide that is an analog of a receptor to an L-ligand comprising:

1) screening at least one L-peptide with a D-ligand that corresponds to said L-ligand, and

2) determining the binding of said at least one L-peptide to said D-ligand.

43. The method of claim 42, further comprising the step of:

3) synthesizing a D-peptide comprising a D-amino acid sequence that corresponds to at least a portion of an L-amino acid sequence of said at least one L-peptide,

wherein said at least one L-peptide binds to said D-ligand

44. The method of claim 42, wherein said L-ligand is a non-peptide with a chiral center.

45. The method of claim 42, wherein said L-ligand is the mirror image of said D-ligand.

46. The method of claim 43, wherein said D-peptide is a D-antibody.

47. The method of claim 42, wherein said at least one L-peptide is at least one L-FAb fragment.

48. The method of claim 47, wherein said at least one L-FAb fragment is phage display library of L-FAb fragments.

49. The method of claim 48, wherein said phage library of L-FAb fragments contains fragments of progressively smaller L-FAb fragments, wherein no F-Ab fragment is less than 6 amino acids and each fragment is identical in amino acid sequence to at least one portion of at least one L-Ab fragment.

50. The method of claim 49, wherein said L-ligand has a molecular weight of a peptide no more than 50 amino acids in length.

51. The method of claim 50, wherein L-FAb fragments have a mutated region 5 to 8 amino acids in length containing selective amino acid replacements.

52. The method of claim 51, wherein said at least one L-peptide is at least 100 L-peptides.

53. The method of claim 42, wherein said at least one L-peptide is a combinatorial library.

54. The method of claim 52, wherein said combinatorial library is a phage display library.

55. The method of claim 52, wherein said phage display library encodes peptides 4 to 20 amino acids in length and each peptide has a mutated region.

56. The method of claim 55, wherein said D-ligand is selected from the group consisting of a ligand for a receptor, a substrate for a binding site on an enzyme, a peptide hormone, a non-peptide hormone, a neurotransmitter, a neurotransmitter analog, a steroid, a steroid analog, a co-factor, and a sugar.

57. The method of claim 53, wherein said combinatorial library comprises L-peptides no more than 10 amino acids in length and no more than 10,000 peptides in complexity.

58. The method of claim 57, wherein said screening step comprises repeating said screening step at a higher stringency conditions to select for higher affinity binding of said L-peptides to said D-ligand.

59. The method of claim 58, wherein said L-peptides have an apparent Kd of 1 μM or less.

60. The method of claim 59, wherein said L-peptides have an apparent Kd of .1 μM or less.

61. The method of claim 60, further comprising the step of:

3) synthesizing a D-peptide comprising a D-amino acid sequence that corresponds to at least a portion of an L-amino acid sequence of said at least one L-peptide,

wherein said at least one L-peptide binds to said D-ligand.

62. A product by the process of one of the claims of 22, 23, 41, 42, 43, or 61.

63. A compound comprising:

D-peptide that has a mirror image that binds to D-polypeptide.

64. The compound of claim 63, wherein said D-peptide can bind to an L-amino acid ligand binding site.

65. The compound of claim 64, wherein said L-amino acid ligand binding site is a biological receptor.

66. The compound of claim 64, wherein the amino acid sequence of said L-amino acid ligand binding site is identical to the amino acid sequence of said D-polypeptide, except that said D-polypeptide is made entirely of D-amino acids.

67. The compound of claim 64, wherein said D-peptide is a D-antibody.

68. The compound of claim 67, wherein said D-peptide an L-FAb fragment.

69. The compound of claim 68, wherein said L-FAb fragment is six to 200 amino acids in length.

70. The compound of claim 64, wherein said D-polypeptide is selected from the group consisting of a receptor, a substrate binding site on an enzyme, an epitope of a receptor that interferes with ligand binding when a receptor antibody is bound, ligand binding site of a receptor, a co-factor binding site on an enzyme and a sugar binding site on a protein.

71. The compound of claim 70, wherein said D-peptide is 6 to 20 amino acids in length.

72. The compound of claim 71, wherein said L-peptides have an apparent Kd of .1 μM or less.

73. A compound comprising:
D-peptide that has a mirror image that binds to D-ligand.

74. The compound claim 73, wherein said D-peptide can bind to an L-ligand.

75. The compound claim 74, wherein said L-ligand is a non-peptide with a chiral center.

76. The compound of claim 75, wherein said L-ligand has a molecular weight of a peptide no more than 10 amino acids in length.

77. The compound of claim 74, wherein said D-peptide is a D-antibody.

78. The compound of claim 77, wherein said D-peptide is an L-FAb fragment.

79. The compound of claim 78, wherein said L-FAb fragment is 10 to 100 amino acids in length.

80. The compound of claim 74, wherein said D-ligand is a ligand for a receptor, a substrate for a binding site on an enzyme, a peptide hormone, a non-peptide hormone, a neurotransmitter, a neurotransmitter analog, a steroid, a steroid analog, a co-factor, and a sugar.

81. The compound of claim 70, wherein said D-peptide is 5 to 30 amino acids in length.

82. The method of claim 72, wherein said L-peptides have an apparent Kd of .01 μM or less.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/16358

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :Please See Extra Sheet.
US CL : 424/130.1; 530/300, 353, 350; 435/4; 436/536, 518
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 424/130.1; 530/300, 353, 350; 435/4; 436/536, 518

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	GUICHARD et al. Antigenic mimicry of natural L-peptides with retro-inversopeptidomimetics. Proc. Natl Acad. Sci. October 1994, Vol. 91, pages 9765-9769, see entire document.	22-62
Y	MILTON et al. Total Chemical Synthesis of a D-Enzyme: The Enantiomers of HIV-1 Protease show Demonstration of Reciprocal Chiral Substrate Specificity. Science, 05 June 1992, Vol. 256, pages 14451448, see entire document.	1-21, 63-82
Y	ZAWADZKE et al. A Racemic Protein. Journal of the American Chemical Society, 1992, Vol. 114, pages 4002-4003, see entire document.	1-21, 63-82

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 NOVEMBER 1996	08 JAN 1997

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	HEATHER BAKALYAR, PH.D.
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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/16358

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	US 5,248,611 A (BENKOVIC ET AL.) 28 September 1993, see entire document.	1-21, 63-82
Y	US 5,079,152 A (BENKOVIC ET AL.) 07 January 1992, see entire document.	1-21, 63-82

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/16358

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/16358

A. CLASSIFICATION OF SUBJECT MATTER:
IPC (6):

A61K 38/00, 39/395; C07K 1/00, 5/00, 7/00, 14/00, 16/00, 17/00; C12Q 1/00; G01N 33/543, 33/536

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

DIALOG: author and word. search terms: (d, dex, dextrorotary) (L ,natural,levorotary) amino acid, peptide, analyte, generat?, assay, antibod?, etc.
databases: medline, uspat, embase, wpi, biosis, scisearch.

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1.

Group I, claim(s)1-21, 63-82, drawn to D-antibody, D-peptide and methods related to said D-antibody and D-peptide.
Group II, claim(s) 22-62, drawn to L-peptide and methods related to said D-peptide.

The inventions listed as Groups I and II do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: D-peptides/antibodies are structurally and functionally distinct from L-peptides/antibodies. Further, because they are distinct in both structure and function, the methods of making and using said D antibody/peptide are different from the methods of making and using said L antibody/peptide and the methods related to one group does not suggest the other.